

IN THE SPECIFICATION

Please replace the Sequence Listing on pages 41-53 of the disclosure, with the attached paper copy of the Substitute Sequence Listing.

Please delete the paragraph at page 3, lines 9-12.

~~Accordingly, the present invention provides an alkaline protease which has excellent detergency against complex soil, exhibits enhanced protein secretion amount and enhanced specific activity, and can be produced at high productivity.~~

Please replace the paragraph beginning on page 6, line 17 and ending on page 8, line 20 with the following replacement paragraph:

Examples of the "alkaline protease having an amino acid sequence of SEQ ID NO: 1" include KP43 {(derived from *Bacillus sp.* KSM-KP43 (FERM BP-6532), Patent Publication WO99/18218)} (SEQ ID NOS: 12 and 13). Examples of the "alkaline protease having an amino acid sequence that exhibits 80% or higher homology with the amino acid sequence of SEQ ID NO: 1" include protease KP9860 (GenBank Accession No. AB046403) {(derived from *Bacillus sp.* KSM-kp9860 (FERM BP-6534), Patent Publication WO99/18218)}; protease 9865 (GenBank Accession No. AB084155) {(derived from *Bacillus sp.* KSM-9865 (FERM P-1592), Japanese Patent Application Laid-Open (*kokai*) No. 2003-199559)} (SEQ ID NOS: 14 and 15; protease E-1 (GenBank Accession No. AB046402) {(derived from *Bacillus* No. D-6 (FERM P-1592), Japanese Patent Application Laid-Open (*kokai*) No. 49-71191)} (SEQ ID NOS: 16 and 17); protease Ya (GenBank Accession No. AB046404) {(derived from *Bacillus sp.* Y (FERM BP-1029), Japanese Patent Application Laid-Open (*kokai*) No. 61-280268)} (SEQ ID NOS: 18 and 19); protease SD521 (GenBank Accession No. AB046405) {(derived from *Bacillus* SD521 (FERM P-11162), Japanese Patent

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Application Laid-Open (*kokai*) No. 3-191781}} SEQ ID NOS: 20 and 21; protease A-1 (GenBank Accession No. AB046406) {{derived from NCIB12289, Patent Publication WO88/01293}}(SEQ ID NOS: 22 and 23); protease A-2 {{derived from NCIB12513, Patent Publication WO98/56927}} (SEQ ID NOS: 24 and 25); mutant proteases described in Japanese Patent Application Laid-Open (*kokai*) Nos. 2002-218989 and 2002-306176; mutants obtained through substitution of position 251 of the amino acid sequence of SEQ ID NO: 1 by asparagine, threonine, isoleucine, valine, leucine or glutamine; mutants obtained through substitution of position 256 of the same amino acid sequence by serine, glutamine, asparagine, valine, or alanine (Japanese Patent Application Laid-Open (*kokai*) 2003-125783); a mutant obtained through substitution of position 65 of the amino acid sequence of SEQ ID NO: 1 by proline; a mutant obtained through substitution of position 101 of the same amino acid sequence by asparagine; mutants obtained through substitution of position 273 of the same amino acid sequence by isoleucine, glycine, or threonine; mutants obtained through substitution of position 320 of the same amino acid sequence by phenylalanine, valine, threonine, leucine, isoleucine, or glycine; mutants obtained through substitution of position 359 of the same amino acid sequence by serine, leucine, valine, isoleucine, or glutamine, mutants obtained through substitution of position 387 of the same amino acid sequence by alanine, lysine, glutamine, glutamic acid, arginine, or histidine (Japanese Patent Application ~~Application~~ Laid-Open (*kokai*) 2004-000122); mutants obtained through substitution of position 163 of the amino acid sequence of SEQ ID NO: 1 by histidine, aspartic acid, phenylalanine, lysine, asparagine, serine, isoleucine, leucine, glutamine, threonine or valine; mutants obtained through substitution of position 170 of the same amino acid sequence by valine or leucine; mutants obtained through substitution of position 171 of the same amino acid sequence by alanine, glutamic acid, glycine, or threonine (Japanese Patent Application ~~Application~~ Laid-Open (*kokai*) 2004-057195); and an alkaline protease having an amino acid

sequence that exhibits a 80% or higher, preferably 87% or more, more preferably 90% or more, still more preferably 95% or more, homology with any of the above listed amino acid sequences.

Please replace the paragraph beginning on the third line from the bottom of page 22- page 23, line 23, with the following replacement paragraph:

A region of about 2.0 kb up to the stop codon of an alkaline protease structural gene derived from *Bacillus sp.* KSM-KP43 was subjected to error prone PCR by use of a Takara Taq (product of Takara), which lacks error repair ability , and by use of adequate amounts of manganese sulfate and dimethylsulfoxide, whereby random mutagenesis was introduced. PCR was carried out using primer 1 (SEQ ID NO: 3 4) and primer 2 (SEQ ID NO: 4 5) capable of amplifying the above mentioned DNA fragment of about 2.0 kb, wherein primer 1 was a sense primer with BamHI linker at the 5' end, and primer 2 was an antisense primer with linker at the 5' end. In PCR, the template DNA was denatured at 94°C for one minute, followed by 30 cycles of treatment, each cycle consisting of 94°C x one minute, 55°C x one minute, and 72°C x two minutes. The amplified DNA fragments were purified by use of a DNA Product Purification kit (Roche), and the terminal restriction endonuclease linkers were cleaved with BamHI and XbaI. The amplified DNA was mixed with plasmid pHA64 which had undergone treatment with BamHI and XbaI (see Japanese Patent Application Laid-Open (kokai) 2000-287687; BamHI- and XbaI-cleaved sites are contained in a downstream region of promoter 64), and subsequently, ligation reaction was carried out with Ligation High (product of Toyobo). Through ethanol precipitation, plasmid was recovered from the ligase reaction mixture. *Bacillus sp.* KSM-9865 (FERM P-18566) serving as the host bacterium was transformed.

Please replace the paragraph beginning at page 12, line 21 to page 13, line 8, with the following rewritten paragraph:

When the alkaline protease of the present invention is a mutant, the alkaline protease before undergoing mutagenesis (which may be referred to as a “parent alkaline protease”) is either a “protease having an amino acid sequence of SEQ ID NO: 1” or the aforementioned “another alkaline protease.” When the parent alkaline protease is subjected to mutation at a predetermined site thereof, the alkaline protease of the present invention can be obtained. For example, when an amino acid residue at a position selected from the aforementioned positions (a) to (g) of the amino acid sequence of SEQ ID NO: 1 of protease (KP43 or an amino acid residue at a position corresponding to any of the above positions in the amino acid sequence of another alkaline protease) is replaced by another amino acid residue, the alkaline protease of the present invention can be obtained.

Please replace the paragraph beginning at page 17, line 21 to page 18, line 2, with the following rewritten paragraph:

Generally, a surfactant is incorporated into the detergent composition in an amount of 0.5 to 60 mass%. In particular, the amount of surfactant is preferably 10 to 45 mass% for preparing a powdery detergent composition, and 20 to 50 mass% for preparing a liquid detergent composition. When the detergent composition of the present invention serves as a bleach composition or a detergent composition for an automated dishwasher, a surfactant is typically incorporated in an amount of 1 to 10 mass%, preferably 1 to 5 mass%.

Please replace the paragraph beginning on page 26, line 20 and ending at the bottom of page 27 with the following replacement paragraph:

Next, in an attempt to combine the above-described individual mutation sites, recombinant PCR was performed through use of primers 1 to 8 (SEQ ID NOs: ~~3 to 4~~ 4 to 11) and Pyrobest (Takara), whereby mutants each bearing combinatorial mutation sites were created. Briefly, using as a template a wild type gene or a mutant gene, a DNA fragment having a size of about 700 bp (including the 15th and 16th positions) from the translation initiation site of the alkaline protease structural gene was amplified with primer 1 (SEQ ID NO: ~~3~~ 4) and primer 3 (SEQ ID NO: ~~5~~ 6) for combinatorial mutation, to thereby create a mutant. Similarly, a DNA fragment having a size of about 500 bp including positions 166 and 167 was amplified with primer 4 (SEQ ID NO: ~~6~~ 7) and primer 5 (SEQ ID NO: ~~7~~ 8); a DNA fragment having a size of 400 bp including position 187 was amplified with primer 6 (SEQ ID NO: ~~8~~ 9) and primer 7 (SEQ ID NO: ~~9~~ 10); and a DNA fragment having a size of about 500 bp (including positions 346 and 405) up to the termination codon of the alkaline protease structural gene was amplified with primer 2 (SEQ ID NO: ~~4~~ 5) and primer 8 (SEQ ID NO: ~~10~~ 11). Respective mutants were incubated and then assessed for protease productivity. Improvement in productivity was confirmed on S15H/S16Q/K346R/N405D, S15H/S16Q/N187S/K346R/N405D, and S15H/S16Q/N166G/G167V/N187S/K346R/N405D. The results show that improvement in productivity was attained by the enhanced amount of secreted protein (107 to 112%) or an improved specific activity (103 to 115%) in respective mutants, on the basis of the secretion amount or the specific activity of a wild type enzyme being taken as 100% (Table 2).

Please replace the paragraph beginning at page 32, lines 4-10, with the following rewritten paragraph:

The slurry (solid content: 50 mass%) was spray-dried with 250° C hot air, to thereby yield a granular base containing sodium polyacrylate (mass average molecular weight:

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10,000) (7 mass%), sodium carbonate (26 mass%), sodium sulfate (20 mass%), sodium chloride (6 mass%), the fluorescent dye (0.5 mass%), zeolite (40 mass%), and water (0.5 mass%).